



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

CNS Invasion of *TCF3::PBX1+* Leukemia Cells Requires Upregulation of AP-1 Signaling As Revealed By Brain Organoid Model

Philip Gebing¹, Stefanos Loizou¹, Sebastian Hänsch, PhD², Julian Schliehe-Diecks¹, Lea Spory³, Pawel Stachura¹, Aleksandra Pandyra⁴, Martin Schrappe, MD⁵, Gunnar Cario, MD⁵, Ameera Alsadeq, PhD⁶, Denis Martin Schewe⁷, Arndt Borkhardt, MD¹, Lennart Lenk, PhD³, Ute Fischer, PhD⁸, Sanil Bhatia, PhD⁴

¹ Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

² Center for Advanced Imaging, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

³ Department of Pediatrics I, Pediatric Haematology/Oncology, ALL-BFM Study Group, University Medical Center Schleswig-Holstein, Kiel, Germany

⁴ Department of Pediatric Oncology, Hematology and Clinical Immunology, Medical faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

⁵ Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany

⁶ Institute of Immunology, Ulm University Medical Centre, Ulm, Germany

⁷ Department of Pediatrics, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

⁸ Department of Pediatric Oncology, Hematology and Clinical Immunology, Heinrich Heine University, Medical Faculty, Düsseldorf, Germany

Introduction:

Involvement of the central nervous system (CNS) remains a challenge in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Currently, the investigation of CNS leukemia mechanisms relies heavily on 2D cell culture and mouse models. However, given the differences between human and murine CNS in cellular identity and architecture, it becomes crucial to explore alternative models to study CNS leukemia. Moreover, novel targets for diagnosis and treatment of CNS ALL are critically needed.

Methods:

In this study, we established a pioneering 3D co-culture model that combines human induced pluripotent stem cell (iPSC)-derived cerebral organoids with BCP-ALL cells. We developed new methods to extract data from our invasion assays by enhancing the visualization of 3D organoid images, enabling us to accurately measure the invasion depth of leukemia cells compared to healthy controls within the organoids relative to their surface. To gain further insights on leukemia cells invading the organoids compared to those in the non-invaded fraction, we conducted RNA sequencing and immunofluorescence staining. Subsequently, we validated these results in a BCP-ALL *in vivo* mouse model and in patients initially diagnosed with CNS-positive BCP-ALL compared to CNS-negative cases within a cohort of 100 BCP-ALL patients.

Results:

Our experiments demonstrated robust and deep engraftment of *TCF3::PBX1+* leukemia cell lines and patient-derived xenograft (PDX) cells into cerebral organoids (within a 14-day of co-culture). In contrast, the engraftment of healthy human CD34+ hematopoietic stem and progenitor cells (HSPCs) was limited ($p < 0.05$) as compared to leukemia cells. Utilizing the co-culture model, we successfully validated the targeting of CNS leukemia-relevant pathways, such as CD79a/Ig α or CXCR4-SDF1, via genetic knockdown or blocking experiments using inhibitor, respectively, which reduced the invasion of BCP-ALL cells into the organoids. Of note, RNA sequencing and immunofluorescence staining analysis revealed a significant upregulation of members of the AP-1 transcription factor complex, namely FOS, FOSB, and JUN, in the organoid-invading cells. Furthermore, we found an enrichment of AP-1 pathway genes in PDX cells recovered from the CNS compared to spleen blasts of mice transplanted with *TCF3::PBX1+* PDX BCP-ALL cells ($n = 5$), thereby supporting the critical role of AP-1 signaling in CNS disease (Figure 1A). In line with these findings, we observed significantly higher levels of the AP-1 gene JUN in patients initially diagnosed with CNS-positive BCP-ALL compared to CNS-negative cases (mean JUN expression: 633.4 ± 78.51 in CNS-negative vs 1142 ± 296.5 in CNS-positive) within a cohort of 100 BCP-ALL patients (Figure 1B).

Summary:

In summary, we present ALL co-culture systems with iPSC-derived cerebral organoids as a promising complementary model to investigate CNS involvement in BCP-ALL including therapeutic targeting approaches, and identified the AP-1 pathway as a marker of CNS disease in *TCF3::PBX1+* BCP-ALL.

Disclosures Cario: Servier, Amgen: Research Funding; JazzPharma: Speakers Bureau.

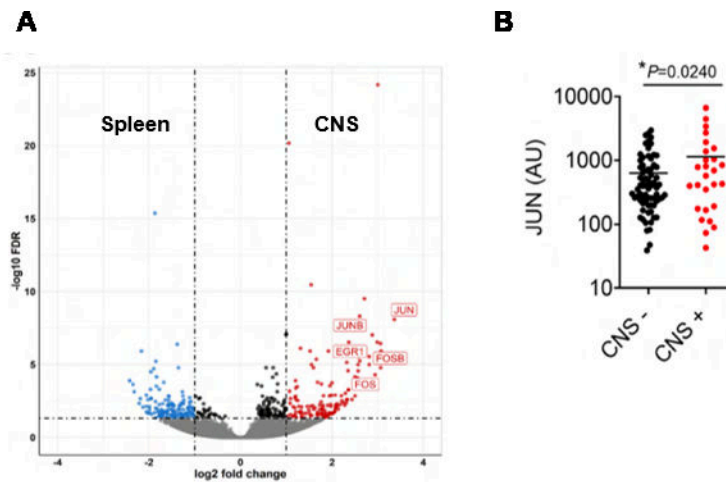


Figure 1: (A) Volcano plot (from RNA-seq data) showing significant ($FDR < 0.05$; $\log_2(FC) < -1$ or $\log_2(FC) > 1$) upregulation of AP-1 member genes in BCP-ALL PDX cells recovered from the CNS compared to PDX cells isolated from the spleen. (B) JUN-expression levels in BCP-ALL patients diagnosed as CNS-positive (CNS +) versus CNS-negative (CNS -), Chi-square-test.

Figure 1

<https://doi.org/10.1182/blood-2023-178613>

Downloaded from http://ashpublications.net/blood/article-pdf/142/Supplement_1/1407/2183406/blood-8010-main.pdf by guest on 08 June 2024